

PATENT  
Attorney Docket No. STG-167  
Customer No. 27,495

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re U.S. Patent Application of

Applicant: Michael BORNS

Application No.: 10/805,650

Filing Date: 19 March 2004

Group Art Unit: 1637

Confirmation No.: 9645

Examiner: M. STAPLES

Title: DNA POLYMERASE FUSIONS AND USES THEREOF

**Mail Stop: RCE**  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

**DECLARATION UNDER 37 C.F.R. §1.132**

I, Michael Borns declare that:

1. I have read U.S. Application No. 10/805,650, filed 19 March 2004, as well as the pending claims (Attachment A).
2. I am the inventor of the subject matter claimed in U.S. Application No. 10/805,650.
3. I am informed and believe that U.S. Application No. 10/805,650 claims the benefit of U.S. Provisional Application No. 60/457,426, filed 25 March 2003.
4. I graduated from the University of Oregon in 1988 with a B.S. in molecular biology. I worked for a year in a Drosophila lab studying the genes and proteins involved in retinal degeneration in the larval development of the fruit fly. From 1989-1990 I worked at Children's

Hospital doing cancer research. In 1990, I started working for a company called Xoma in Santa Monica, California as a research associate. At Xoma my research focused on cloning genes, and I performed standard molecular biology experiments, such as PCR, Southern blotting, and Northern blotting. I left Xoma around 1992 and started working for One Lambda in Canoga Park, California as a senior research associate. I worked at One Lambda for five years studying DNA polymerases, such as Taq, and developing PCR-based tissue typing kits. In 1997, I changed jobs and started working for Stratagene in San Diego, California as a senior research associate. I am currently employed by Stratagene, an Agilent Technologies Company, and hold the title of research scientist expert. During my ten years at Stratagene, I have worked in the enzyme group, studying DNA polymerases, including Pfu polymerase, and developing PCR kits.

5. The statements made in this declaration are based on my 15 years experience working with DNA polymerases, including the last 10 years working with the wild type Pfu DNA polymerase.

6. I have read Item 12 (pages 6-11) of the Office Action mailed 17 August 2007 and understand that the Examiner has rejected certain claims as being unpatentable over "Wang" (WO 01/082501).

7. As of at least 25 March 2003, the pH of the buffering component in the standard PCR reaction buffers for wild type Pfu DNA polymerase was 8.3 to 8.8.

8. In general, when the buffering component is added to the PCR reaction buffer, the final pH of the PCR reaction buffer will be slightly lower than the pH of the buffering component.

9. With standard wild type DNA polymerases, such as wild type Pfu DNA polymerase, as pH increases above 9, there is an inverse relationship with PCR performance. In other words, as the pH increases above 9, PCR performance decreases.

10. Using a wild type Pfu DNA polymerase in a reaction buffer having a pH above 9 impairs the efficiency of PCR performance.

11. Attachment B shows the results of an experiment I performed in October 2007 where I examined the effects of increasing pH on wild type Pfu DNA polymerase (Pfu Turbo) for the amplification of a 6 kb human beta globin DNA target. Lane 1 is a 1 kb DNA marker. Lanes 2, 3, and 4 are with pH 8.3, 8.5, and 8.8, respectively. Lanes 5 and 6 are with pH 9.5 and 10, respectively.

12. In this experiment, a 6 kb human globin genomic DNA target sequence was amplified with 2.5 units of wild type Pfu DNA polymerase and 100 ng human genomic DNA per 50  $\mu$ l reaction in each lane. A 1 minute per kb extension time was used for a total extension time of 6 minutes.

13. As shown in Attachment B, wild type Pfu DNA polymerase efficiently amplifies target DNA in a reaction buffer ranging from pH 8.3 to 8.8. However, when the pH of the reaction buffer was increased above 9, the efficiency of the Pfu amplification activity dropped

dramatically. The results in Attachment B are consistent with my experience working with wild type Pfu DNA polymerase as of 25 March 2003.

14. In contrast, with the DNA polymerase fusions of this invention, increasing the pH of the reaction buffer above 9 actually enhances—not reduces—the efficiency of PCR performance.

15. I have read the Wang reference cited in the 17 August 2007 Office Action. Example 6.1 of Wang discloses using wild type Pfu polymerase and a Pfu-Sso7d fusion polymerase with a reaction buffer containing a 20 mM Tris-HCl buffering component having a pH of 8.8. It appears that the reaction buffer used by Wang was the commercially available Pfu reaction buffer from Stratagene.

16. Based on my experience working with wild type Pfu DNA polymerase, I would have expected as of at least 25 March 2003 that increasing the pH of the Pfu reaction buffer disclosed in Example 6.1 of Wang above a pH of 9 would significantly impair the conditions for amplification with the wild type Pfu DNA polymerase. Without considering any of the work disclosed in U.S. Application No. 10/805,650, and based solely on my experience working with wild type Pfu DNA polymerase, I would have similarly expected as of at least 25 March 2003 that increasing the pH of the Pfu reaction buffer disclosed in Example 6.1 of Wang above a pH of 9 would significantly impair the conditions for amplification with Wang's Pfu-Sso7d fusion polymerase. Therefore, as of at least 25 March 2003, I would not have been motivated to increase the pH of the reaction buffer disclosed in Example 6.1 of Wang as a matter of routine optimization for either the wild type Pfu DNA polymerase or the Pfu-Sso7d fusion polymerase,

because I would have expected that doing so would significantly impair the efficiency of their PCR performance. In addition, as of 25 March 2003, I would not have been motivated to increase the pH of the reaction buffer disclosed in Example 6.1 of Wang as a matter of routine optimization because Wang appears to have been using the commercially available Pfu reaction buffer from Stratagene, which as a commercial product, would have already been optimized.

17. The expectations referred to in the preceding paragraph of my declaration are based solely on my experience working with wild type Pfu DNA polymerase and other wild type DNA polymerases and do not take into account any of the work disclosed in the instant application, which was not publicly available as of the 25 March 2003 filing date of U.S. Provisional Application No. 60/457,426.

18. Thus, it was surprising when I discovered that increasing the pH of the reaction buffer above 9 enhances, rather than impairs, the PCR performance efficiency of the DNA polymerase fusions in U.S. Application No. 10/805,650, and blends comprising the same. *See* Figures 1-9 of the '650 application. This unexpected result was duly noted in the application. For example, the application notes that "PCR reactions using the high pH 10.0 and 11.8 reaction buffers were dramatically superior to the 1.5X cloned *Pfu* buffer, further demonstrating the enhancing effects of high pH for PCR amplification with *Pfu*-Sso7d (figure 3)." U.S. Application No. 10/805,650 at pages 80-81.

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19. The undersigned further declares that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing therefrom.

Dated: 12/17/07

By:



Michael Borns

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# ATTACHMENT A

**PENDING CLAIMS**

1. (Previously presented) A method for DNA synthesis at high pH, comprising: a) contacting a DNA polymerase fusion with a nucleic acid template under conditions of high pH, and b) effecting template dependent synthesis of DNA, wherein said high pH ranges from 9.3 to 14, and wherein said DNA polymerase fusion comprises wild type *Pyrococcus furiosus* polymerase I fused to *Sulfolobus solfataricus* SSso7d protein, wherein said DNA polymerase fusion functions as a DNA polymerase.

2. (Previously presented) The method of claim 1, further comprising contacting a PCR enhancing factor and/or an additive with said DNA polymerase fusion and said nucleic acid template.

3. (Previously presented) A method for the cloning of a DNA synthesis product, at high pH, wherein said high pH ranges from 9.3 to 14, comprising:

a) providing a DNA polymerase fusion comprising wild-type *Pyrococcus furiosus* polymerase I fused to *Sulfolobus solfataricus* SSso7d protein, wherein said DNA polymerase fusion functions as a DNA polymerase;

b) contacting said DNA polymerase fusion with a nucleic acid template under conditions of said high pH so as to effect the template dependent synthesis of a DNA synthesis product, and

c) inserting said synthesized DNA product into a cloning vector, thereby cloning said synthesized DNA product.

4. (Previously presented) The method of claim 3, further comprising contacting a PCR

enhancing factor and/or an additive with said DNA polymerase fusion and said nucleic acid template in step (b).

5. (Previously presented) A method for sequencing DNA at high pH, wherein said high pH ranges from 9.3 to 14, comprising the steps of:

(a) contacting a template DNA strand with a sequencing DNA primer;

(b) contacting said DNA of step (a) with a DNA polymerase fusion comprising wild-type *Pyrococcus furiosus* polymerase I fused to *Sulfolobus solfataricus* SSso7d protein, wherein said DNA polymerase fusion functions as a DNA polymerase, deoxyribonucleoside triphosphates, and a chain-terminating nucleotide analog;

(c) incubating the mixture of step (b) under conditions sufficient to synthesize at said high pH a random population of DNA molecules complementary to said DNA molecule, wherein said synthesized DNA molecules are shorter in length than said first DNA molecule and wherein said synthesized DNA molecules comprise a terminator nucleotide at their 5' termini; and

(d) separating said synthesized DNA molecules by size so that at least a part of the nucleotide sequence of said first DNA molecule can be determined.

6. (Original) The method of claim 5, further comprising a PCR enhancing factor and/or an additive.

7. (Previously presented) A method of linear or exponential PCR amplification at high pH, wherein said high pH ranges from 9.3 to 14, for site-directed or random mutagenesis comprising the step of: incubating a reaction mixture comprising a nucleic acid template, at least

one PCR primers, and a DNA polymerase fusion comprising wild-type *Pyrococcus furiosus* polymerase I fused to *Sulfolobus solfataricus* SSso7d protein, wherein said DNA polymerase fusion functions as a DNA polymerase, under conditions which permit amplification of said nucleic acid template at said high pH by said fusion to produce a mutated amplified product.

8. (Original) The method of claim 7, further comprising a PCR enhancing factor and/or an additive.

9. (Previously presented) A method of reverse transcriptase PCR at high pH, wherein said high pH ranges from 9.3 to 14, comprising the step of incubating a reaction mixture comprising a nucleic acid template, at least one PCR primer, and a DNA polymerase fusion comprising wild-type *Pyrococcus furiosus* polymerase I fused to *Sulfolobus solfataricus* SSso7d protein, wherein said DNA polymerase fusion functions as a DNA polymerase, under reaction conditions which permit amplification of said nucleic acid template at said high pH by said fusion to produce an amplified product.

10. (Original) The method of claim 9, further comprising a PCR enhancing factor and/or an additive.

11. (Cancelled)

12. (Withdrawn) The method of claim 11, wherein said DNA polymerase fusion comprises a Glycine to Proline substitution at amino acid position 387 (G387P) and has reduced DNA polymerization activity.

13. (Previously presented) The method of claim 1, 3, 5, 7 or 9, wherein said DNA

polymerase fusion comprises reduced base analog detection activity relative to wild-type *Pyrococcus furiosus* polymerase I under identical reaction conditions.

14. (Withdrawn) The method of claim 13, wherein said DNA polymerase fusion comprises reduced base analog detection activity and a mutation at position V93, wherein said mutation is a Valine to Arginine substitution, a Valine to Glutamic acid substitution, a Valine to Lysine substitution, a Valine to Aspartic acid substitution or a Valine to Asparagine substitution.

15. (Previously presented) The method of claim 11 wherein said DNA polymerase fusion has reduced base analog detection activity relative to wild-type *Pyrococcus furiosus* polymerase I under identical reaction conditions.

16. (Withdrawn – previously presented) The method of claim 12, wherein said DNA polymerase fusion comprises reduced base analog detection activity relative to wild-type *Pyrococcus furiosus* polymerase I under identical reaction conditions.

17. (Withdrawn) The method of claim 11, wherein said DNA polymerase fusion further comprises a mutation at position V93, wherein said mutation is a Valine to Arginine substitution, a Valine to Glutamic acid substitution, a Valine to Lysine substitution, a Valine to Aspartic acid substitution or a Valine to Asparagine substitution that confers a reduced base analog detection activity phenotype to said chimeric DNA polymerase.

18. (Withdrawn) The method of claim 12, wherein said DNA polymerase fusion further comprises a mutation at position V93, wherein said mutation is a Valine to Arginine substitution, a Valine to Glutamic acid substitution, a Valine to Lysine substitution, a Valine to Aspartic acid

substitution or a Valine to Asparagine substitution that confers a reduced base analog detection activity phenotype to said chimeric DNA polymerase.

19. (Cancelled)

20. (Cancelled)

21. (Withdrawn) The method of claim 13, wherein said DNA polymerase fusion further comprises a Glycine to Proline substitution at amino acid position 387 (G387P) that confers a reduced DNA polymerization phenotype to said chimeric DNA polymerase.

22. (Withdrawn) The method of claim 14, wherein said DNA polymerase fusion further comprises a Glycine to Proline substitution at amino acid position 387 (G387P) that confers a reduced DNA polymerization phenotype to said chimeric DNA polymerase.

23. (Withdrawn) The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion further comprises an Aspartate to alanine substitution at amino acid 141 (D141A) and a Glutamic acid to Alanine substitution at amino acid position 143 (D141A/E143A) that renders said chimeric DNA polymerase 3'-5' exonuclease deficient.

24. (Withdrawn) The method of claim 13, wherein said DNA polymerase fusion with reduced base analog detection activity further comprises an Aspartate to alanine substitution at amino acid 141 (D141A) and a Glutamic acid to Alanine substitution at amino acid position 143 (D141A/E143A) that renders said chimeric DNA polymerase 3'-5' exonuclease deficient.

25. (Original) The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion comprises a wild type, mutant or chemically modified DNA polymerase.

26. (Previously presented) The method of claim 1, 3, 5, 7, or 9, wherein said DNA polymerase fusion is a proofreading polymerase.

27. (Previously presented) The method of claim 26, wherein said proofreading polymerase comprises wild-type *Pyrococcus furiosus* polymerase I.

28. (Previously presented) The method of claim 1, 3, 5, 7, or 9, wherein said DNA polymerase fusion comprises an increase, as compared to said wild type *Pyrococcus furiosus* polymerase I, in at least one activity selected from the group consisting of: processivity, proofreading, fidelity, DNA binding activity, strand displacement activity, polymerase activity, nucleotide binding and recognition, efficiency, template length amplification capability, GC-rich target amplification efficiency, specificity, thermostability, intrinsic hot start capability, or salt resistance.

29. (Previously presented) The method of claim 1, 3, 5, 7 or 9, wherein said DNA polymerase fusion comprises a reduction, as compared to said wild type *Pyrococcus furiosus* polymerase I, in at least one activity selected from the group consisting of: amplification slippage on templates with tri-nucleotide repeat stretches, extension time in a PCR reaction or amplification cycles in a PCR reaction.

30. (Cancelled)

31. (Withdrawn – previously presented) A kit for performing at high pH, wherein said high pH ranges from 9.3 to 14, a method selected from the group consisting of: DNA synthesis; cloning of a DNA synthesis product; sequencing DNA; RT PCR; and linear or exponential PCR

amplification comprising a DNA polymerase fusion and packaging materials therefore.

32. (Withdrawn – previously presented) The kit of claim 31, further comprising a high pH buffer, wherein said high pH buffer has a pH which ranges from 9.3 to 14.

33. (Withdrawn) The kit of claim 31, further comprising a PCR enhancing factor and/or an additive.

34. (Withdrawn – previously presented) A composition for any one of DNA synthesis, cloning of a DNA synthesis product at high pH, sequencing DNA, linear or exponential PCR amplification for site directed or random mutagenesis, RT-PCR comprising a DNA polymerase fusion and a high pH buffer, wherein said high pH buffer has a pH which ranges from 9.3 to 14.

35. (Withdrawn – previously presented) A composition for DNA synthesis, comprising a DNA polymerase fusion and a high pH DNA synthesis buffer, wherein said high pH DNA synthesis buffer has a pH which ranges from 9.3 to 14.

36. (Withdrawn – previously presented) A composition for cloning of a DNA synthesis product, comprising a DNA polymerase fusion and a high pH DNA cloning buffer, wherein said high pH cloning buffer has a pH which ranges from 9.3 to 14.

37. (Withdrawn – previously presented) A composition for sequencing DNA, comprising a DNA polymerase fusion and a high pH DNA sequencing buffer, wherein said high pH sequencing buffer has a pH which ranges from 9.3 to 14.

38. (Withdrawn – previously presented) A composition for linear or exponential PCR amplification for site directed or random mutagenesis, or for RT-PCR comprising a DNA

polymerase fusion and a high pH PCR reaction buffer, wherein said high pH PCR reaction buffer has a pH which ranges from 9.3 to 14.

39. (Withdrawn) The composition of claims 34, 35, 36, 37 or 38, further comprising a PCR enhancing factor and/or an additive.

40. (Previously presented) The method of any one of claims 1, 3, 5, 7, and 9, wherein said DNA polymerase fusion is encoded by SEQ ID NO: 126 and has an amino acid sequence of SEQ ID NO:127.

41. (Previously presented) The method of claim 29, wherein the activity is extension time in a PCR reaction.

42. (New) The method of claim 1, wherein said high pH ranges from 9.5 to 12.

43. (New) The method of claim 3, wherein said high pH ranges from 9.5 to 12.

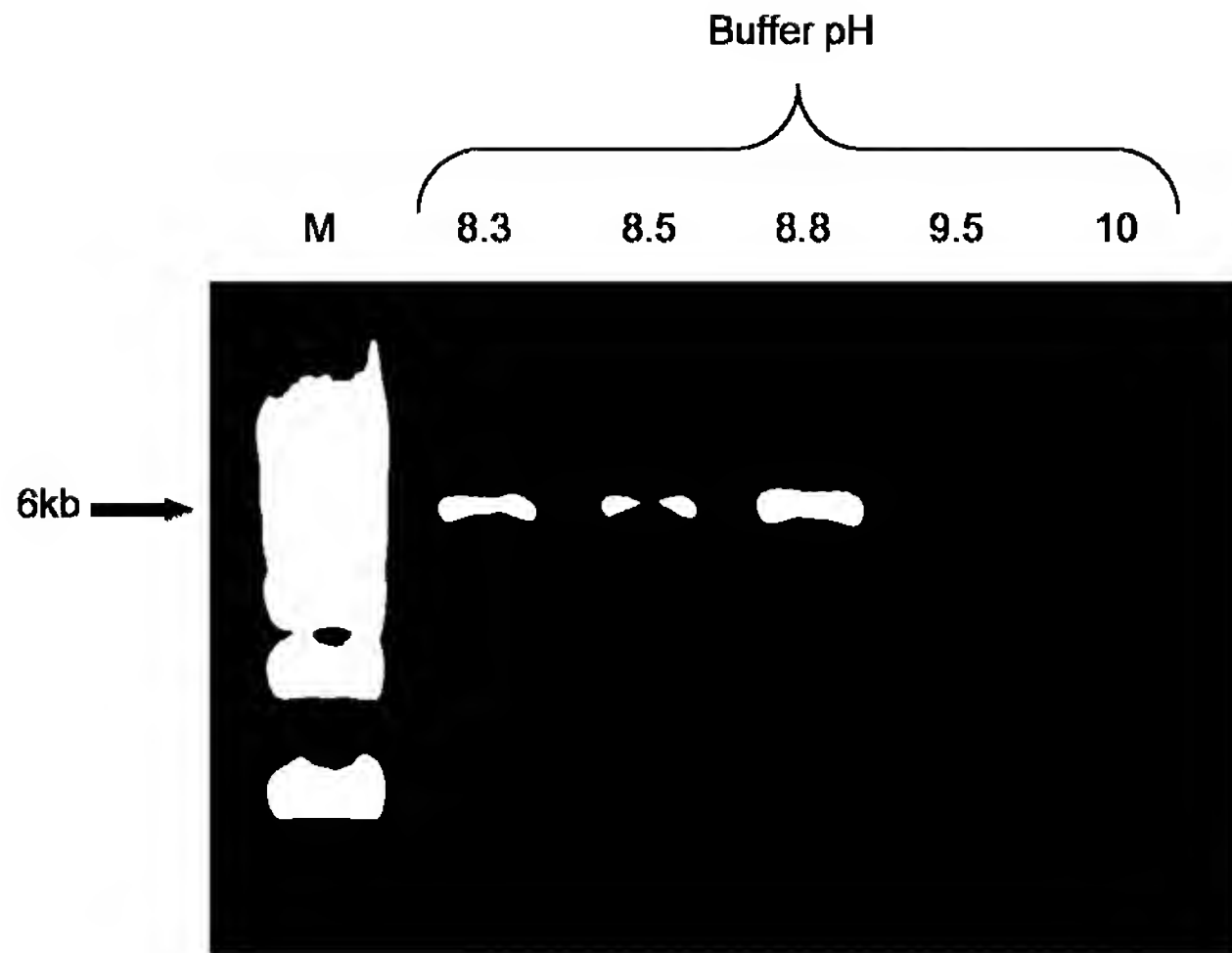
44. (New) The method of claim 5, wherein said high pH ranges from 9.5 to 12.

45. (New) The method of claim 7, wherein said high pH ranges from 9.5 to 12.

46. (New) The method of claim 9, wherein said high pH ranges from 9.5 to 12.

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# ATTACHMENT B



- 6kb human beta globin genomic DNA target (100ng human genomic DNA per 50 $\mu$ l reaction)
- 2.5 units of *PfuTurbo* per 50 $\mu$ l reaction
- 1 minute per kb extension time (6 minute total extension time)
- M (1kb ladder DNA marker)